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1. (Amended) The composition of claim 40 wherein the buffer comprises 50% (volume/volume) glycerol[,]; 20 mM Tris-HCl, pH 8[,]; 0.1 mM ethylenediamine tetraacetic acid[,]; 1 mM dithiothreitol[,]; 0.5% volume/volume of a polyoxyethylated sorbitan monolaurate[,]; 0.5% volume/volume of an ethoxylated nonyl phenol[,]; and 200 µg/ml gelatin.

Remarks

The Invention

The present invention provides stabilized thermostable nucleic acid polymerase compositions. Since 1987 and due to the benefits provided by the present application, thermostable nucleic acid polymerases have come into widespread use. Suppliers that formulate their thermostable nucleic acid polymerase with the stabilizers of the present invention have met with great commercial success. These stabilizers, non-ionic polymeric detergents, help maintain polymerase activity in purified preparations of thermostable nucleic acid polymerase during subsequent or later use in the heating and cooling steps of the PCR or in DNA sequencing applications.

Prior to the present invention, non-ionic detergents were used in solubilizing membranes and in purifying proteinaceous macro-molecules including enzymes. Detergents serve in those processes to permeabilize or lyse viruses or cells, dissociate aggregates, and facilitate chromatographic means of separation and purification. Prior to Applicants' invention of the claimed compositions, it was unknown that purified thermostable polymerases require a detergent stabilizer to maintain enzyme activity upon storage. Prior to Applicants' invention, it was unknown that the presence of non-ionic detergent in the storage buffer would serve to stabilize enzyme activity. The claimed invention was novel and non-obvious at the time the invention was made, and Applicants' patent application was first filed. Many have followed Applicants' lead and copied the invention first disclosed by Applicants in their patent application. Applicants deserve patent protection for their invention and request the Examiner to consider the declarations filed herewith and the remarks herein.

In the Claims

Applicants have amended Claim 1 to more clearly describe the claimed invention in response to Examiner's concerns under 35 U.S.C. §112, second paragraph, as discussed below. Applicants have amended Claims 40 and 41 to correct grammatical errors indicated by the Examiner. As amended, the claims more clearly describe that "pH 8.0" is not a component, but

rather a characteristic of the element "Tris-HCl." The amendments are supported by the specification, and Applicants request the Examiner to enter the amendments.

The Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1, 35-39, and 53-65 are rejected under 35 U.S.C. §112, first paragraph, because the Examiner believes that the disclosure is enabling only for claims limited to a buffer as required by Claim 40. The Examiner refers to the M.P.E.P. at §706.03(n) and §706.03(z) and asserts that the "specification (page 79, lines 10-14) discloses only this buffer as a specific buffer that can be used."

Applicants respectfully traverse the rejection and point to the specification as originally filed for support. At page 24, beginning at line 21, in the section entitled "Stabilization of Enzyme Activity," Applicants fully describe and enable the invention of Claim 1. The specification, at that section, characterizes elements of the claimed invention with sufficient detail and enumeration to provide a vast array of embodiments. For the Examiner's convenience, this section of Applicants' specification is reproduced below.

For long-term stability, the enzyme herein must be stored in a buffer that contains one or more non-ionic polymeric detergents. Such detergents are generally those that have a molecular weight in the range of approximately 100 to 250,000, preferably about 4,000 to 200,000 daltons and stabilize the enzyme at a pH of from about 3.5 to about 9.5, preferably from about 4 to 8.5. Examples of such detergents include those specified on pages 295-298 of McCutcheon's Emulsifiers & Detergents, North American edition (1983), published by the McCutcheon Division of MC Publishing Co., 175 Rock Road, Glen Rock, NJ (USA), the entire disclosure of which is incorporated herein by reference. Preferably, the detergents are selected from the group comprising ethoxylated fatty alcohol ethers and lauryl ethers, ethoxylated alkyl phenols, octylphenoxy polyethoxy ethanol compounds, modified oxyethylated and/or oxypropylated straight-chain alcohols, polyethylene glycol monooleate compounds, polysorbate compounds, and phenolic fatty alcohol ethers. More particularly preferred are Tween 20, from ICI Americas Inc., Wilmington, DE, which is a polyoxyethylated (20) sorbitan monolaurate, and Iconol™ NP-40, from BASF Wyandotte Corp. Parsippany, NJ, which is an ethoxylated alkyl phenol (nonyl).

The thermostable enzyme of this invention may be used for any purpose in which such enzyme is necessary or desirable. In a particularly preferred embodiment, the enzyme herein is employed in the amplification protocol set forth below.

Thus, Applicants' disclosure specifically provides the support for the claimed invention as follows:
(1) the disclosure provides a generic characterization of the suitable and preferred properties of non-

ionic polymeric detergents for stabilization of the enzyme; (2) no less than nine classes of non-ionic polymeric detergents are enumerated as being preferred detergents for practicing the claimed invention; (3) two detergents are identified by chemical type and commercial trade name as being most preferred; and (4) a publication naming additional detergents is cited and incorporated by reference.

In making the rejection, the Examiner refers to the M.P.E.P. §706.03(n), which states, in part:

In chemical cases, a claim may be so broad as to not be supported by disclosure, in which case it is rejected as unwarranted by the disclosure.

However, the present case meets and exceeds any reasonable standard gleaned from §706.03(n). If the enumeration of at least 11 species of the claimed invention are judged insufficient, Applicants request the Examiner to show the basis for such a standard in the law so that Applicants can readily comply.

The rejection also refers to the M.P.E.P. at §706.03(z), which describes the necessary support for obtaining broad generic claims given the disclosure of a single species. The scope of claims for inventions in the chemical arts is particularly addressed where it is not obvious from the disclosure of one species, what other species will work.

It is well settled that in cases involving chemicals and chemical compounds, which differ radically in their properties, it must appear in an Applicant's specification either by the enumeration of a sufficient number of the members of a group or by other appropriate language, that the chemicals or chemical combinations included in the claims are capable of accomplishing the desired result.

(M.P.E.P. at 706.03[z]) (emphasis added). The present application enumerates a sufficient number of members of a group and appropriate language to describe chemicals included in the claims capable of accomplishing the desired result. The language includes a description of chemical properties of compounds capable of accomplishing the desired result, particularly at page 24 beginning at line 21. Thus, the specification meets the standard required by the sections of the M.P.E.P. cited by the Examiner in the rejection under 35 U.S.C. §112, first paragraph.

The Examiner indicates that only the buffer of Claim 40 is enabled, because "the specification (page 79, lines 10-14) discloses only this buffer as a specific buffer that can be used." Yet in the specification, Applicants describe that those particular components exemplify a preferred embodiment of the invention. The specification does not suggest, at page 79, lines 10-14, that the

buffer disclosed is the only buffer that can be used. Applicants have previously described that the particular buffer used is not an essential or critical feature of the invention. The crux of Applicants' claimed invention is the presence of a non-ionic detergent for stabilizing enzyme activity. Thus, Applicants intend Claim 1 to describe "a purified thermostable nucleic acid polymerase in a buffer that comprises" a non-ionic detergent as a stabilizer. Claim 1 has been amended to reflect Applicants' intent.

The best mode requirement is met by Applicants' description of their most preferred embodiment of the invention at the time the application was filed. In view of the amendment to Claim 1 and breadth of the disclosure, including the recitation of other exemplary compounds for stabilizing the thermostable enzyme, there is no basis under the law for the Examiner's assertion that the scope of the claimed invention must be limited to Applicants' best mode embodiment. Applicants respectfully request the Examiner to reconsider and withdraw the rejection of Claims 1, 35-39, and 53-65 under 35 U.S.C. §112, first paragraph.

The Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 40 and 41 are rejected under 35 U.S.C. §112, second paragraph, as being confusing and unclear because the Examiner finds "pH 8.0" is listed as a component of the claimed buffer. Applicants have amended Claims 40 and 41 in response. The amendment serves to clarify that "pH 8.0" is not a component but rather a characterization of the buffering agent Tris-HCl.

In view of the amendments to Claims 40 and 41, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of Claims 40 and 41 under 35 U.S.C. §112, second paragraph.

The Rejection of Claims 1, 35-39, and 53-59 Under 35 U.S.C. §102(a)

The Examiner has rejected Claims 1, 35-39, and 53-59 as being anticipated by the MBR product information sheet. The Examiner relies on the Office Actions dated September 4, 1990, and May 3, 1991, in the parent application, U.S. Serial No. 07/387,003, for an explanation of the reasons for the rejection.

A. The Declaration Under 37 C.F.R. §1.131 Dated April 21, 1992

In a Preliminary Amendment filed in the present application on April 24, 1992, Applicants submitted a Rule 131 declaration signed by all named inventors. The Examiner has taken the position that the declaration fails to establish priority.

The Examiner has reviewed the Rule 131 declaration filed April 24, 1992, the attachments, Applicants' specification, and the cited reference and identified differences between these documents as contributing to Examiner's conclusion that Applicants' declaration fails to establish priority. At page 3 of the Office Action, the Examiner has questioned Applicants' assessment that the non-ionic detergent is responsible for stability of the enzyme. Further, the Examiner suggests that "other components could be essential for effective stability."

Applicants respectfully assert that the issues raised in the Office Action stray from the relevant concerns under 35 U.S.C. §102(a). The question under that statute is whether or not the invention was described in a printed publication prior to the invention thereof by Applicants. The remarks in the outstanding Office Action suggest that the Examiner fails to understand and questions the operability of the disclosed and claimed invention. Applicants welcome the opportunity to discuss these issues if the Examiner so requires; however, at present, Applicants will respond directly to the specific rejections of the claims. Applicants response to those rejections is guided by statute and the M.P.E.P.

Accordingly, Applicants request the Examiner to withdraw the rejection of Claims 35-39 and 53-59 under 35 U.S.C. §102(a) in view of the declaration under 37 C.F.R. §1.131 previously supplied. The declaration does not establish a date for recognizing that the non-ionic detergent alone serves to stabilize the thermostable nucleic acid polymerase. However, there is no requirement under the law for Applicants to antedate a reference by showing when Applicants recognized the benefits afforded by their invention. To mandate such a requirement would demand that Applicants demonstrate a date of conception. This is not the proper test for considering Applicants' Declaration under 37 C.F.R. §1.131.

Applicants have prepared and submitted their Rule 131 Declaration in accordance with the law. Rule 131 requires Applicants to "make oath to facts showing a completion of the invention" before the effective date of the reference. Analogous issues have been previously decided by the courts.

In In re Moore the cited reference did not disclose or suggest any utility for the claimed compound. In that case the Applicant attempted to overcome the reference and filed a Rule 131 Affidavit which contained no showing of a utility for the compound. The P.T.O. rejected on the grounds of Brenner v. Manson, 383 U.S. 519, 148 U.S.P.Q. 689 (1966). The C.C.P.A. reversed and found that the P.T.O. was applying standards applicable to a priority contest in an interference proceeding rather than ex parte prosecution. Judge Balwin speaking for the C.C.P.A.

held the affidavit sufficient, and citing In re Wilkinson (50 C.C.P.A. 701, 134 U.S.P.Q. 171 (1962)), stated:

The basis of the Wilkinson decision was simply, that an applicant need not be required to show any more acts with regard to the subject matter claimed than can be carried out by one of ordinary skill in the pertinent art following the description contained in the reference. The discovery or identification of a chemical compound and the determination of how to make it are sufficient inventive acts with regard to that compound. Under the Wilkinson rationale, the third inventive act (i.e., the determination of a practical utility when one is not obvious) need not have been accomplished prior to the date of a reference unless the reference also teaches how to use the compound it describes. That case holds that an applicant may be compelled to prove only that he had prior possession of the thing itself in order to remove a reference which shows no more to the public.

In re Moore, 58 C.C.P.A. 1340, 444 F.2d 572, 578-580, 170 U.S.P.Q. 260 (1971) (emphasis added).

The standard described in In re Wilkinson and reiterated in In re Moore must be applied in the present case. Applicants' 131 Declaration demonstrates possession of the claimed invention and all that is provided by MBR product information sheet prior art to June 8, 1987. Consequently, under the law, the MBR reference is not suitable for rejecting Applicants' claims under 35 U.S.C. §102(a).

B. The Declaration Under 37 C.F.R. §1.131 Dated January 6, 1993

Applicants respectfully assert that the previously supplied Declaration Under 37 C.F.R. §1.131 is sufficient under the law for antedating the MBR reference cited by the Examiner, and Applicants are prepared to pursue this matter on Appeal. However, in the interest of expediting allowance of the pending claims, a further declaration is supplied coincident with this response. This Declaration, signed by all of the named inventors, and appended exhibits, demonstrates that Applicants had conceived of and reduced to practice that which is now claimed, prior to the publication date of the cited MBR reference.

Additionally, Applicants respectfully direct the Examiner's attention to Exhibit C at page 101 where co-inventor Saiki describes that the purified enzyme preparation that was observed to continually lose enzyme activity with the passage of time did, in fact, contain gelatin. The experiments described in the Exhibits demonstrated that the addition of non-ionic detergent to that purified enzyme composition restored and stabilized enzyme activity. Thus, the Declaration not only serves to antedate the MBR product information sheet but proves that gelatin and non-ionic

detergents do not have an equivalent effect on enzyme activity. These facts are further supported by the Akers Declaration particularly at Experiment 3 where gelatin and non-ionic detergent are compared for their effects, or lack thereof, on the enzyme activity of a purified thermostable DNA polymerase. Gelatin is not a storage buffer stabilizer for a purified thermostable polymerase.

C. The Additional References

In the rejection under 35 U.S.C. §102(a), the Examiner discusses the combination of Goff et al. or Spiegelman and Feller et al. with the MBR disclosure. Applicants note that the combination is improper under §102(a); however, Applicants believe that the Examiner is suggesting that these secondary references describe the state of the art at the time for reviewing the MBR disclosure. The Examiner states at page 4 of the Office Action:

It is clear from the patents that use of the detergent for maintaining enzyme activity was known prior to supplying MBR with the Cetus protocol.

The remarks demonstrate the Examiner's acknowledgement that, in fact, Cetus provided MBR with the detergent buffer described in the disclosure prior to the MBR publication. The rejection cannot stand where the Examiner has accepted the facts alleged in the Rule 131 Declaration, which facts demonstrate Applicants prior reduction to practice. Further, the secondary references do not demonstrate that detergent was known for maintaining enzyme activity. Indeed, none of the references deal with thermostable enzymes or thermostable DNA polymerases. The facts provided by those references will be discussed fully under the section of this amendment entitled "The Rejection Under 35 U.S.C. §103 where the Examiner has cited Goff et al., Spiegelman, and Feller et al. as secondary references."

Applicants' claimed invention is a stabilized enzyme composition comprising a purified thermostable polymerase enzyme and non-ionic detergent. Applicants invented their invention prior to providing their protocol to MBR and prior to MBR disclosing Applicants' proprietary storage buffer to the public in violation of contractual obligations. Applicants respectfully request the Examiner to withdraw the rejection of Claims 1, 35-39, and 53-59 under 35 U.S.C. §102(a) in view of the remarks herein, the 131 Declaration and Exhibits filed April 24, 1992, the 131 Declaration and Exhibits filed herewith, and the law set forth.

The Rejection of Claims 60-62 Under 35 U.S.C. §103

The Examiner has rejected Claims 60-62 under 35 U.S.C. §103 as being unpatentable over the product information sheet of MBR for reasons set forth in the previous Office Actions of September 4, 1990, and May 3, 1991, in the parent application.

Claims 60 and 61 are dependent claims, and both depend, indirectly, from Claim 1. Claims 60 and 61 recite particular thermostable polymerase enzymes in the stable enzyme composition of Claim 1. Claim 62 is an independent claim that recites an amplification reaction mixture that further comprises the composition of Claim 1.

The Examiner relies on the remarks in the previous and present rejections of Claims 1, 35-39, and 53-59 without further comment. Applicants respectfully traverse the rejection and rely on their discussion of the MBR reference presented above and the April 24, 1992, and January 6, 1993, Declaration Under 37 C.F.R. §1.131 to antedate and remove the reference. Consequently, Applicants request the Examiner to reconsider and withdraw the rejection of Claims 60-62 under 35 U.S.C. §103 over the product information sheet of MBR.

The Rejection Under 35 U.S.C. §103

It is well settled that in considering obviousness under 35 U.S.C. §103, the prior art as a whole must be considered and its teachings must be viewed as they would have been by one of skill in the art at the time of the invention. To properly support a rejection based upon prima facie obviousness, the Examiner must cite a combination of prior art references which sets forth the necessary elements of the claimed invention and which provides the motivation for combining those elements to yield the claimed invention. See, e.g., Northern Telecom Inc. v. Datapoint Corp., 15 U.S.P.Q. 1321, 1323 (Fed. Cir. 1990). If either the necessary elements of the invention or the motivation to combine such elements is missing, the Examiner cannot properly support the rejection based upon 35 U.S.C. §103, and it must be withdrawn.

In a response to a prima facie case of obviousness, Applicants can either respond by rebutting the rejection with argument or by traversing the rejection with a showing of unexpected results or comparative test results. In rebuttal of the Examiner's prima facie case of obviousness, Applicants respectfully submit, as will be explained below, that the cited references fail to identify necessary elements of the present invention, e.g., purified thermostable polymerase enzymes; thus, none of these references can be cited by the Examiner as teaching or suggesting Applicants' claimed compositions. Furthermore, in traversal of the prima facie obviousness rejection, Applicants respectfully submit that the Declarations previously provided under 37

C.F.R. §1.131 demonstrate the surprising and unexpected results and advantages achieved by the invention, which are not found in or obvious from the prior art. The evidence provided by the Gelfand and Akers Declarations must be given appropriate weight and considered fairly in accordance with the law.

Claims 1, 35-39, 53-59, and 62 are rejected under 35 U.S.C. §103 as being unpatentable over Kaledin *et al.* (1980) in view of Goff *et al.* and if necessary, in further view of Feller *et al.* or Spiegelman. In the July 15, 1992, Office Action the Examiner refers to previous Office Actions dated September 4, 1990, and May 31, 1991, for an explanation of the reasoning of the rejection. For clarity and convenience the Examiner's previous assertions regarding the prior art and the claimed invention are reproduced below:

[i]t would have been obvious to store the polymerase of Kaledin *et al.* in a buffer containing a nonionic detergent in view of Goff *et al.* disclosing (col. 8, line 24) that a nonionic detergent is required in recovering this enzyme and if needed in further view of Feller *et al.* (col. 5, line 7) or Spiegelman (col. 6, line 25) disclosing use of a detergent-containing buffer in relation to this type of enzyme.

See Office Action of September 4, 1990, page 6.

A. Elements of the Claimed Invention are Missing

1. The Primary Reference: Kaledin *et al.* (1980)

For the reasons set forth below, Applicants respectfully submit that the presently claimed stabilized compositions comprising a non-ionic detergent and a purified thermostable polymerase enzyme are not obvious in view of the references cited. The Kaledin *et al.* (1980) enzyme is either a crude preparation of a partially characterized and proteolytically degraded enzyme or an entirely different enzyme. The pending claims, however, are directed to a purified enzyme composition. The Patent Office has previously determined that the purified enzyme compositions provided by the present application are novel and non-obvious in view of the crude partially degraded preparations of Kaledin *et al.* (see U.S. Patent No. 4,889,818, which issued from the grandparent to the present application).

Thus, the reasoning that "it would have been obvious to store the polymerase of Kaledin *et al.* in a buffer containing non-ionic detergent" is immaterial, because the polymerase of Kaledin *et al.* is not the polymerase described in the pending claims. The rejection is not supported by the references where none provides or suggests an essential element of the pending claims. None of the references provide or suggest a purified thermostable polymerase enzyme. Without this

element, the references, alone or in combination, do not obviate the claimed invention under 35 U.S.C. §103.

Applicants have previously pointed out that subsequent publications of Kaledin *et al.* refute the earlier, Kaledin *et al.* (1980), description that gelatin is required. In Kaledin *et al.* (1981), cited elsewhere in the Examiner's rejection, the authors show that, although the final preparation of their crude enzyme did not contain gelatin, the preparation was reported to be stable for at least three years without appreciable loss of activity (see Applicants' amendment dated February 8, 1991, in parent application U.S. Serial No. 387,003, at page 7).

The Examiner is required to consider all of the evidence for what it would provide or suggest to one of ordinary skill in the art. Kaledin *et al.* (1980 and 1981) do not obviate the claimed invention.

2. The Secondary References

The secondary references disclose methods for detecting and/or purifying reverse transcriptase. None of the references relate to methods for stabilizing a purified enzyme, as the Examiner asserts. None of the references teach that gelatin and non-ionic detergents are interchangeable, as the Examiner asserts. None of the references relate to purified thermostable polymerases.

The Goff *et al.* reference describes methods of constructing, expressing in *E. coli* and isolating modified forms of Molony (M) Murine Leukemia Virus (MuLV) reverse transcriptase (RT) activity (the product of the M-MuLV pol gene).

Goff *et al.* chose to lyse harvested bacterial cells with lysozyme and non-ionic detergent. Since the Goff expression system directs the accumulation of an intracellular and not extracellular protein product, the recombinant cells are harvested and then must be disrupted or lysed (see column 2, line 45; line 62, line 67; column 7, lines 9-14; column 11, lines 67-68; column 16, lines 12-13; column 17, lines 3-6, emphasis added). No guidance is provided in the specification for choosing this particular method of *E. coli* cell disruption over numerous other methods (eg, lysozyme treatment and repeated cycles of freezing and thawing, sonication, French Press, Virtis homogenizer, blending or grinding with glass beads, Microfluidizer or Matin-Gaulin mill). The person of ordinary skill would simply recognize that the *E. coli* cell walls were digested by the enzyme lysozyme, and then the bacterial membranes were disrupted with non-ionic detergent (eg. NP40).

The choice of lysozyme treatment of bacterial cell walls followed by detergent disruption of bacterial membranes to effect E. coli cell lysis provides no relevant teaching for a stable enzyme composition comprising a purified thermostable DNA polymerase and one or more non-ionic detergents.

Goff et al. chose to address a fundamental problem of insoluble expressed proteins in their expression system primarily by genetic modification and secondarily by detergent and high salt solubilization. The challenges addressed by Goff et al. are entirely different from the instant invention, and the Goff et al. specification provides no teaching or basis to apply the Goff et al. teachings to the invention of non-ionic detergent stabilizers for purified, thermostable DNA polymerases.

Goff et al. describe in detail the construction and properties of three different recombinant plasmids used to direct the expression of M-MuLV RT-like activity or M-MuLV RT-related polypeptides in E. coli.

The first plasmid, pSH1 (Fig 1, top and column 7, lines 41-53), encodes a 120,000 dalton (column 2, lines 67-68) fusion protein comprising 326 codons of the trpE gene fused in-frame to about 810 codons of the central region of the M-MuLV pol gene. When Goff et al. assayed extracts of pSH1-containing E. coli cells, they detected a novel RT-like activity and attributed this activity to the presence and expression of the cloned M-MuLV cloned DNA segment (Table 1, column 8, column 9). Unfortunately, "Virtually all of this polypeptide (the 120,000 dalton fusion protein) was recovered in the insoluble fraction" (column 10, lines 35-37, emphasis added).

Thus, the pSH1-encoded fusion protein was insoluble even in the presence of non-ionic detergent, which, in addition, was unstable since lower than expected molecular weight forms were also seen. To recover any soluble activity from the total crude E. coli cell extract apparently required proteolysis (column 11, lines 39-43) as well as non-ionic detergents and high salt treatment (column 8, lines 23-25). No reasonable extrapolation can be made between the behavior of a hybrid fusion protein containing 326 amino acids of E. coli trpE protein that is covalently linked to 87.7 kDa M-MuLV RT that requires some non-defined proteolysis to derive partially soluble proteins in a total crude extract of E. coli and the instant invention.

To improve the expression level of M-MuLV RT-like activity, improve the stability of the activity, and "to form smaller protein products that would more closely resemble the authentic enzyme" (column 7, lines 54-55), Goff et al. generated an amino-terminal deletion of their pSH1 plasmid (Fig. 1; column 3, lines 1-8; column 7, line 54 through column 8, line 4). In this context, "stability" refers to reduced or no intracellular proteolysis, the i.e., absence of intracellular

proteolytic degradation. "Stability" does not refer to preservation of the activity of a purified protein. Consequently, genetic manipulations were used to remove about 315 codons or about 35,000 daltons. One plasmid, pSHNB6, was characterized extensively.

Goff *et al.* state "a higher proportion of the full sized pSHNB6 protein was recovered in the soluble fraction than with the corresponding pSH1 protein" (column 11, lines 48-50, emphasis added). However "the majority of the fusion proteins partitioned into the insoluble fraction after cell lysis" (column 18, lines 2-3, emphasis added), and "the major product was broken down into smaller species; partial purification of the soluble reverse transcriptase also indicated that multiple species were active" (column 17, lines 63-66).

Goff *et al.* indicate that partial purification of both pSH1 and pSHNB6 proteins was achieved with DEAE-cellulose column chromatography. The activity was bound to the DEAE-cellulose column in low ionic strength and eluted from the column with 0.2 M NaCl (column 7, lines 20-23; column 12, lines 5-8). The specification is silent on and does not indicate any requirement for non-ionic detergent in the elution buffers. Further, the specification is silent on and does not indicate a requirement for non-ionic detergent to solubilize or stabilize the RT activity expressed from plasmid pSHNB6. Accordingly, the skilled practitioner would conclude that the increased expression level as well as the increased stability and improved solubility were the result of the extensive genetic manipulations of the plasmid and not the result of some non-described effects of non-utilized detergents. None of the teachings shed any light on the use of non-ionic detergents as stabilizers of purified thermostable DNA polymerases activity.

To achieve higher expression levels and increased "stability" of expressed proteins (i.e., absence of intracellular, proteolytic breakdown products), Goff *et al.* continue their genetic manipulations of the plasmids encoding M-MuLV RT-like activity. The entire second half of the Goff reference (columns 13-28) describes the modification of plasmid pSHNB6 to obtain plasmid pB6B15.23, characterization of the plasmid and the products expressed by the modified plasmid. Goff *et al.* turned their efforts to the C-terminal domain of the now reduced-in-size novel hybrid tripartite fusion protein: "deletion of this terminus might result in the formation of a protein that more closely resembles the authentic cleavage product and might improve its stability in E. coli" (column 18, lines 7-10). Again, the "stability" to which Goff *et al.* refer is reduced or decreased intracellular breakdown/proteolysis of the primary translation product of their novel hybrid tripartite fusion gene.

The resulting plasmid, pB6B15.23, directed the synthesis of (1) 3.5-4 times more RT activity in E. coli crude extracts (column 16, line 36) than the parent plasmid pSHNB6 and (2) a

new single 71,000 dalton polypeptide that was at least 30% soluble (column 18, line 42) and that was highly stable, over produced (column 16, lines 40-41) and reacted with antibody to reverse transcriptase (column 19, lines 42-45). A careful reading of the reference fails to clarify an apparent contradiction or dichotomy between the partitioning of "the majority" of the RT activity to the insoluble fraction with the parent plasmid and "at least 30% . . . in the soluble fraction" for the improved plasmid. The former statement fails to specify how much more than 50% was in the insoluble fraction and as a result how much less than 50% was soluble. The latter statement fails to specify and the authors decline to show data indicating how much more than 30% is soluble and correspondingly how much less than 70% continued to partition to the insoluble fraction, even in the presence of non-ionic detergent.

Goff et al. note several similarities between the novel tripartite hybrid fusion protein encoded by plasmid pB6B15.23 and authentic M-MuLV reverse transcriptase: the presence of inherent RNaseH activity (columns 20-21); sedimentation as a monomer in glycerol gradients (column 12, lines 36-38); and optimal conditions for synthesis on poly (rA):oligo dT (column 27, lines 57-59).

However, the reference also reveals several differences between the novel hybrid tripartite fusion MuLV RT-like protein encoded by plasmid pB6B15.23 and both authentic M-MuLV reverse transcriptase and the RT-like activity encoded by plasmid pSHNB6. Since the primary amino acid sequence of the novel hybrid tripartite fusion protein encoded by plasmid pB6B15.23 is distinctly different from authentic M-MuLV RT, it is not surprising that differences could exist. For example, column 20, lines 24-26 indicates the requirement during purification for non-ionic detergent "to prevent aggregation (presumably with other cellular E. coli proteins) and loss of activity" as well as the striking 15-fold difference between the ratio of Mn⁺⁺ to Mg⁺⁺ activation on poly (rA):oligo dT templates (column 27, lines 62-68). In addition, whereas the pSHNB6 activity was able to bind to DEAE-cellulose in low salt (see above), the pB6B15.23 activity failed to bind to DEAE-cellulose in low salt (column 20, lines 33-36). Similarly, Goff et al. did not include non-ionic detergents during DEAE chromatography of the pSHNB6 activity (see above), but inclusion of 0.1% nonidet P40 in the DEAE column and elution buffers (column 3, lines 59-62 and column 17, lines 12-16) during purification of the pB6B15.23 activity failed to prevent the loss of almost 80% of the applied activity and concomitant almost 300% decrease in the specific activity (Table 3, cf. 62.7 U/mg for DEAE load vs 21.9 U/mg for DEAE flow-through).

Thus, a careful reading and understanding of the Goff et al. reference not only fails to find support for the Examiner's position but in addition demonstrates that where the various RT fusion

products designed and expressed by Goff *et al.* are so distinct from one another in solubility characteristics, proteolytic stability, and how each is affected by the presence of non-ionic detergent during purification, the reference can provide no clear guidance for suggesting the presently claimed invention. The distinguishing features of each RT-like enzyme engineered by Goff *et al.* supports Applicants' argument that the properties of RT enzymes cannot predict the properties of purified thermostable DNA polymerases. Taken as a whole, Goff *et al.* offers nothing to support the rejection.

The Examiner cites Spiegelman as a secondary reference for showing the use of "a detergent containing buffer in relation to this type of enzyme" (see Office Action, dated September 4, 1990, at page 6). However, the relationship between the buffer and enzyme of Spiegelman is not the relationship between the non-ionic detergent and purified enzyme described and claimed in Applicants' specification.

The Spiegelman reference describes a method to detect breast cancer in human subjects in which the presence of a viral-specific reverse transcriptase activity or protein indicates the presence of a particular type of RNA tumor virus. The reverse transcriptase is a virus-associated enzyme and is found within the viral particles. Spiegelman describes a method for processing breast tumor tissue to isolate "the density region in which RNA tumor viruses localize" (column 5, line 52 to column 6, line 8). To disrupt the viral particles and free or solubilize the reverse transcriptase enzyme contained therein, Spiegelman uses DTT, KCl and non-ionic detergent (column 6 lines 18-21 and column 7, lines 54-55). The use of detergent in Spiegelman relates to lysis of viral particles to free the virion contents. There is no suggestion, however remote, that the use of detergent in this reference relates to (1) stabilization of enzyme activity, (2) purified enzymes of any type, or (3) thermostable polymerases.

Lastly, the Examiner cited Feller *et al.* at column 5, line 7, for disclosing the "use of a detergent containing buffer on relation to this type of enzyme." Like Spiegelman, Feller describes a method for diagnosing breast cancer in mammals by detecting a reverse transcriptase-type of protein in the milk of human patients. The Feller reference discloses methods to enrich for "high density particles . . . from human milk" (column 4, lines 9-22). These "particles contain a lipid rich membrane coat surrounding a central "core" containing nucleic acid plus the reverse transcriptase plus other enzymes. The preparation of the enzyme thus comprises removal of the lipid rich membrane followed by breaking up of the core, separation of the nucleic acid therefrom and final purification of the enzyme" (column 4, lines 23-29). Specifically, Feller indicates that the detergent is used for "breaking up the core" (column 4, lines 56-61). Feller indicates the nucleic acid from

the disrupted, high density viral particles is then removed by the first chromatographic column using DEAE-cellulose (column 4, line 64 through column 5, line 11).

As with Spiegelman, the Feller reference does not describe purified thermostable enzyme preparations or the relationship of non-ionic detergent to enzyme activity for purified enzyme preparations. All three secondary references are silent on stabilization of purified enzymes. All four references cited in the rejection are silent on purified thermostable polymerases.

The rejection made in the September 4, 1990, Office Action and reiterated in the outstanding Office Action cannot stand because the disclosures cited offer nothing alone or in combination for teaching, suggesting, or motivating the claimed invention. In view of the foregoing, it is readily apparent that none of the references cited by the Examiner teach or suggest all of the necessary elements of the claimed invention; none of these references suggest the claimed compositions comprising a purified thermostable enzyme and at least one non-ionic detergent. Absent any such teaching or suggestion in the prior art, the claimed stabilized enzyme compositions are non-obvious and, thus, patentable.

B. The References Provide No Motivation or Suggestion to Combine to Provide the Claimed Invention

Applicants have reviewed the art cited in the rejection and pointed out that the references fail to provide the essential elements of the claimed invention. Assuming arguendo that the Examiner disagrees, Applicants further point out that there is no suggestion to combine the cited references. A rejection based on a primary reference showing a crude preparation of partially degraded Thermus aquaticus polymerase described as stabilized with gelatin, and three references for preparing reverse transcriptase using detergent to lyse cells or viral particles, or to facilitate chromatography, is insufficient.

The Examiner relies on Kaledin *et al.* (1981) to reject Claims 60 and 61, yet selectively overlooks that the reference teaches that the thermostable DNA polymerase is stable for three years without gelatin (page 1250 at section 5). This selective combination, relying only on particular portions of the references to reject the pending claims, is not suggested by the cited disclosures and is inappropriate.

The law is clear; there must be a suggestion or motivation to combine the references and that combination must place the claimed invention into the hands of the skilled artisan. The present rejection does not meet this test and should be withdrawn.

C. Surprising Results and Comparative Data: The Decision Maker Must Start Over

In the Office Action dated May 3, 1991, the Examiner asserts at page 4 that Kaledin et al. teach the use of gelatin to stabilize their enzyme and acknowledges that the reference does not provide or suggest the claimed invention; however, the Examiner believes that the secondary references, combined with Kaledin et al., render Applicants' invention obvious:

when Goff et al. and, if needed, Feller et al. or Spiegelman are considered, it would have been apparent that in addition to gelatin a nonionic detergent would have also functioned to stabilize the polymerase. To use an alternative stabilizing agent known for polymerase stabilization would have been a matter of obvious choice depending on individual preference and convenience. Furthermore, to select a preferred stabilizing agent from these known would have required only limited routine experimentation and have been within the skill of the art.

The Examiner's assertion regarding the obviousness of a choice between gelatin and non-ionic detergent as a stabilizer for a thermostable nucleic acid polymerase based on the disclosure of Goff et al. was refuted by the evidence presented in the Rule 132 Declarations of James Akers and David Gelfand. These Declarations accompanied Applicants' Preliminary Amendment filed April 24, 1992, in the present application. In the Office Action mailed July 15, 1992, the Examiner states that the Akers and Gelfand Declarations are unpersuasive, and the previous rejection is maintained. Applicants respectfully traverse the rejection and request the Examiner, as required by law, to consider the Declarant's evidence.

1. The Akers Declaration

The Examiner discounts the evidence provided in the Akers Declaration and concludes at page 5 of the Office Action that because the result of Akers conflicts with the Goff et al. reference the Declaration is unpersuasive:

There is seen no reason to accept this declaration result as correct and the result of Goff et al. as incorrect.

This subjective approach to an analysis of obviousness is inappropriate, and Applicants request reconsideration. The C.C.P.A. has held that:

When prima facie obviousness is established and evidence is submitted in rebuttal, the decision maker must start over. Though the burden of going forward to rebut the prima facie case remains with the Applicant, the question of whether that burden has been successfully carried requires that the entire path to decision be retraced. An earlier decision should not, as it was here, be considered as set in concrete, and Applicants' rebuttal evidence then be evaluated only on its knockdown ability. Analytical fixation on an earlier decision can tend to provide that decision with an unreservedly broadened umbrella effect. Prima facie obviousness is a legal conclusion not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which the earlier conclusion was reached, not against the conclusion itself."

(emphasis added) In re Rinehart, 189 U.S.P.Q. 143, 531, F.2d 1048 (C.C.P.A. 1976)

The analytical approach mandated by the C.C.P.A. is not evidenced by the Examiner's remarks in the present Office Action. Rather, the Examiner has taken the approach of examining only the "knockdown" value of the declaration and dismissed the new facts because they present a conflict with the Examiner's earlier conclusion regarding obviousness.

The law requires that the evidence must be reviewed anew. Accordingly, Applicants refer to the Examiner's rejection based on Goff et al., relied on in the present Office Action and articulated in the Office Action dated September 4, 1990, at page 6.

It would have been obvious to store the polymerase of Kaledin et al. in a buffer containing a non-ionic detergent in view of Goff et al. disclosing (column 8, line 24) that a non-ionic detergent is required in recovering this enzyme.

In view of the evidence Applicants have provided in the Rule 132 Declaration and the guidance provided by the C.C.P.A., Applicants request the Examiner to reconsider the rejection.

At column 8, in the section of Goff et al. indicated by the Examiner, the reference states:

The level of activity per ml in these crude extracts was considerably higher than that in viral harvests taken from infected NIH/3T3 cell lines. Recovery of the activity in the soluble fraction required the presence of non-ionic detergent and high salt concentrations.

The Goff et al. reference describes a method for constructing, expressing, and isolating modified forms of reverse transcriptase activity. Partial recovery of enzyme activity from soluble enzyme fragments cannot be expanded to teach that a purified enzyme requires one or more non-ionic detergents for stabilization of enzyme activity as described by Claim 1. The claimed invention

cannot be found obvious in view of a reference describing different compositions and a different function where neither the claimed composition nor the claimed function are suggested.

The disclosure of Goff et al. and the Akers Declaration do not conflict as Examiner asserts. The conflict is in the extrapolation of what the Examiner believes Goff et al. could supply to the art. It is this extrapolation of the reference that is in conflict with the evidence.

The Akers Declaration does not dispute that a non-ionic detergent is required for recovery of reverse transcriptase activity from a crude extract; that fact, as provided by Goff et al., is not questioned or tested by the experimental evidence provided and attested to. However, in the rejection at page 5 the Examiner asserts that Goff et al. discloses that a non-ionic detergent prevents loss of enzyme activity. This hindsight interpretation of Goff et al. appears to be the basis of the rejection under 35 U.S.C. §103. Goff et al. do not discuss enzyme stability, prevention of activity loss, or storage buffers. "Stability" referred to in Goff et al. pertains to prevention of proteolytic degradation of fusion proteins, either intracellular or during lysis and preparation of crude extracts. Clearly, the Examiner has interpreted the need expressed in Goff et al. for including detergent in the preparation of a crude extract as being equivalent to providing storage stability of enzyme activity of a purified enzyme. The experiments described in the Akers Declaration were designed to test this assertion that a crude extract is equivalent to a purified enzyme.

At Experiment 1, the Akers Declaration examines whether or not non-ionic detergent prevents loss of enzyme activity as Examiner suggests is taught by Goff et al. The Declaration demonstrates that for a purified reverse transcriptase composition, enzyme activity is not effected by the presence or absence of non-ionic detergent in the storage buffer. Thus, the Akers Declaration does not conflict with the Goff et al. reference, because Goff et al. do not describe stability of enzyme activity for purified reverse transcriptase compositions. The Akers Declaration, however, does conflict with the Examiner's prima facie obviousness rejection based on Goff et al. Appropriately, the choice of enzyme for Experiment 1 was reverse transcriptase, the enzyme described in the reference cited for teaching non-ionic detergent in relation to enzymes.

The Examiner dismisses Experiment 2 of the Akers Declaration and again asserts that while the Declaration demonstrates that a non-ionic detergent is required for stability of a purified thermostable DNA polymerase, this result "is expected since Goff et al. suggest that a non-ionic detergent prevents loss of activity" (Office Action at page 6). The Examiner is using hindsight to review and interpret the Goff et al. reference in view of Applicants' specification. This is impermissible by law. There can be no other explanation for the Examiner's remarks because Goff et al. do not ever mention that "non-ionic detergent prevents loss of activity." Only Applicants

have addressed this concern. Goff et al. describe purification and characterization of recombinant fusion proteins as full length and fragmented products; storage stability of enzyme activity is not discussed anywhere in the reference. Goff et al. is silent on the stability of purified enzyme activity.

Experiment 1 demonstrates that the enzyme which is the subject of the Goff et al. disclosure does not support the Examiner's proposition that non-ionic detergent prevents loss of purified reverse transcriptase enzyme activity.

Experiment 1 taken with Experiment 2 demonstrates that the Examiner's proposition that all polymerase enzymes are equivalent is erroneous. The Examiner states in the May 31, 1991, Office Action, relied on in the present rejection, at page 5:

There is seen nothing to lead one to believe that a polymerase being thermostable changes its characteristics to as to make unexpected a non-ionic detergent functioning to stabilize the enzyme as suggested by Goff et al.

The comparative data provided by Experiments 1 and 2 of the Akers Declaration refutes the Examiner's assertion and must be considered. The disclosure of Goff et al. must be considered for what that disclosure factually provides and not for what the Examiner gleans in hindsight.

The Examiner is concerned that the experimental conditions do not all find a counterpart within the pending claims and states at page 6 of the Office Action:

[i]n all the experiments a standard buffer of a certain pH was used that contained substances not required by the present claims.

Applicants find the remarks confusing. The claimed invention is a stabilized enzyme composition and not an assay mixture. The claimed composition is a component of the experiments described in the Akers Declaration. If the Examiner is suggesting that the invention as claimed is inoperative, Applicants request the Examiner to describe the rejection more clearly so that Applicants can fully respond.

At page 6 of the Office Action the Examiner objects to the Akers Declaration, because for Experiments 2 and 3, NP-40 and Tween 20 were used, which the claims do not require. Applicants note that the claims require at least one non-ionic detergent. Thus, the combination of Tween 20 and NP-40 is within the scope of Claim 1. The Board of Appeals has determined that when making a comparison with prior art compounds, there is no need to include in the tests compounds not exemplified in the prior art, and the Applicant is fully justified in testing the closest

compounds actually taught in the reference (Ex parte Westphal, 223 U.S.P.Q. 630 (Bd. App. 1983). Accordingly, the non-ionic detergents noted in the purification scheme of Goff et al. (column 6, line 65, and column 7, line 14) are included for comparative testing.

At Experiment 3, the Akers Declaration demonstrates that gelatin is not equivalent to non-ionic polymeric detergent for effecting the activity of Taq DNA polymerase. Applicants note that in the Office Action the Examiner did not comment on the evidence of the Akers Declaration at Experiment 3, but continued to assert that gelatin and non-ionic detergent are interchangeable and obvious as choices. Examiner has yet to provide any reference that supports this assertion. The Akers Declaration refutes the assertion of equivalence and must be considered. The Declaration filed herewith under 37 C.F.R. §1.131 provides further evidence at Exhibit D that gelatin and non-ionic detergent are not equivalent, and gelatin is ineffective for stabilizing purified thermostable polymerase enzymes.

2. Gelfand Declaration

The Declaration of co-inventor Gelfand submitted in accordance with 37 C.F.R. §1.132 was provided with Applicants' Preliminary Amendment filed April 24, 1992. The Declaration was prepared to provide factual evidence for traversing the Examiner's rejection of the pending claims as obvious over Kaledin et al. (1980) in view of Goff et al. However, the Examiner discounts the Declaration and states:

the Rule 132 Declaration of Gelfand is unpersuasive since there is seen nothing to support that a difference in amino acid sequence and isoelectric point influences how a non-ionic detergent effects enzyme activity.

The Akers Declaration at Experiments 1 and 2 demonstrates that the properties of MuLV RT are not predictive of thermostable DNA polymerases. Thus, the Akers Declaration compares stability characteristics of a reverse transcriptase with a thermostable DNA polymerase.

The Gelfand Declaration refutes the Examiner's assertion that all polymerase enzymes would be expected to have equivalent properties relating to stability and the effect of gelatin and non-ionic detergents. The Gelfand Declaration compares the physical properties of reverse transcriptase and a thermostable DNA polymerase.

The Gelfand Declaration demonstrates that the reverse transcriptase enzyme described in the cited prior art and the thermostable enzymes of the invention are unrelated compounds. The Declaration shows that reverse transcriptase is more closely related to a random amino acid

sequence than it is related to Tag polymerase. The question then is not, as Examiner has asked, what enzyme characteristics make it unpredictable; but what in the cited references supports the Examiner's assertion that the properties of one enzyme are predictive of another unrelated enzyme.

Applicants assert that the prior art cited does not support the rejection, and this is proved by the facts attested to in the Akers and Gelfand Declarations. Consequently, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of Claims 1, 35-39, 53-59 and 62 under 35 U.S.C. §103 over Kaledin et al. (1980) and Goff et al. in view of the remarks herein, the Akers and Gelfand Declarations, and the law.

D. The Prior Art Teaches Away From the Claimed Invention

Applicants respectfully assert that the rejection under 35 U.S.C. §103 cannot stand because the claimed invention is not provided, suggested, or motivated by the combination of references proposed by the Examiner. The Patent Office must view the references fairly for what each provides. Further, the Patent Office must consider prior art that teaches away from the claimed invention as evidence of patentability. Applicants provide a copy of Wu and Cetta, 1975, Biochemistry 14(4):789-795, coincident with this response.

Wu and Cetta report that non-ionic detergents stimulate viral reverse transcriptase. The article presents a comparison of the effects of non-ionic detergent on bacterial and mammalian DNA polymerases and states in the abstract:

The detergent stimulation appears to be specific for viral reverse transcriptase since this effect is not observed with purified bacterial DNA polymerase or with three known mammalian cellular DNA polymerases.

Wu and Cetta suggest that the contrasting effect of detergent on polymerases purified from viral, bacterial and mammalian sources is useful as a criterion for distinguishing viral reverse transcriptase from other DNA polymerases.

E. coli polymerase I, the Klenow fragment of E. coli pol I, T₄ DNA polymerase, and T₇ DNA polymerases do not require non-ionic detergent in the storage buffer. Applicants provide copies of mesophilic DNA polymerase product descriptions copied from current catalogs of major commercial molecular biology reagent suppliers: BioLabs, Stratagene, Promega, and Pharmacia. Storage and/or shipping buffers are highlighted for Examiner's convenience. Examiner will note that none include non-ionic detergent in the storage buffer. One of ordinary skill in the art would conclude that a thermostable DNA polymerase from a thermophilic microorganism would certainly

not require the presence of non-ionic detergent to preserve activity at $< 4^{\circ}\text{C}$. Applicants' invention is particularly surprising since E. coli pol I and Thermus aquaticus or T. thermophilus (Tth) DNA polymerase are far more closely related in amino acid sequence than Taq or Tth and M-MuLV RT.

It has long been a criterion of patentability that there is an invention present when the teachings and express expectations of the prior art are contradicted. In United States v. Adams, the Supreme Court reaffirmed this fundamental principle and found patentable an invention where the inventor went against accepted teachings that would deter investigation (U.S.P.Q. 479, 383 U.S. 39 (1966)). The prior art cited in the present rejection is insufficient for supporting the Examiner's assertion that the invention is an obvious substitution of apparent equivalents. As in United States v. Adams, the rejection is refuted by presenting factors which would have led away from, rather than to, the substitution.

The Rejection of Claims 60 and 61

The Examiner has rejected Claims 60 and 61 under 35 U.S.C. §103 as being unpatentable over the references as applied to Claims 1, 35-39, 53-59, and 62 and further in view of Kaledin et al. (1981).

In the Office Action dated September 4, 1990, at page 7, in rejecting Claims 60 and 61 under 35 U.S.C. §103, the Examiner states:

when using a detergent containing buffer for polymerase, as set forth above, it would have been a matter of obvious choice to use polymerase from the organism disclosed by Kaledin et al. (1981) or Ruttimann et al. (1985).

Claim 60 depends indirectly from Claim 1 and is drawn to a stable enzyme composition comprising a non-ionic detergent and purified thermostable DNA polymerase isolated from the species Thermus flavus. Claim 61 is analogous to Claim 60, wherein the thermostable DNA polymerase is isolated from the species Thermus thermophilus.

In the outstanding rejection of Claims 60 and 61 under 35 U.S.C. §103, the Examiner has removed Ruttimann et al. as a reference. Accordingly, Applicants will confine their remarks to the rejection as stated in the July 15, 1982, Office Action, at page 6, restated above. Further, Applicants rely on the discussion of Kaledin et al. (1980) and Goff et al. provided in the preceding pages of the present amendment to refute the rejection of Claims 60 and 61. The additional reference in the rejection of Claims 60 and 61, Kaledin et al. (1981) is briefly addressed below.

Kaledin et al. (1981) describe the isolation of a thermoactive DNA polymerase from Thermus flavus. The Examiner suggests that Kaledin et al. (1981) provides an obvious choice for a polymerase in a detergent containing buffer. However, the "choice" provided by that reference cannot support a prima facie obviousness rejection of claims where the reference neither suggests or motivates the claimed invention. The Kaledin et al. (1981) reference would serve only to teach away, not towards, the claimed invention, because the reference describes that the enzyme did not lose appreciable activity when stored for three years without gelatin. Further, non-ionic detergent is not present, yet the enzyme preparation is reported as stable. One of ordinary skill in the art would find nothing in Kaledin et al. (1981) to motivate the invention recited in Claim 60 or 61.

Kaledin et al. (1981), therefore, adds nothing to the rejection of Claims 60 and 61, and Applicants rely on the arguments herein and Declarations previously provided to traverse the rejection under 35 U.S.C. §103. Consequently, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of Claims 60 and 61 under 35 U.S.C. §103, in view of Kaledin et al. (1980), in view of Goff et al. and further in view of Kaledin et al. (1981).

Conclusion

Applicants have amended the claims and provided supportive evidence in accordance with 37 C.F.R. §1.131 and §1.132 to place the present application condition for allowance, in view of the forgoing and the remarks herein. Applicants respectfully request favorable consideration of the application, withdrawal of the Examiner's rejections, and the issuance of a formal Notice of Allowance.

The Commissioner is hereby authorized to charge any associated fees in connection with this amendment to Deposit Account No. 08-2525.

If the Examiner believes that a telephone conferences would expedite prosecution of this application, please telephone the undersigned at (510) 814-2863.

Respectfully submitted,

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